31. The method according to claim 30, wherein said viral infection of herpes groups is an Epstein-Barr virus infection.

32. The method according to claim 14, wherein said patient is virally infected, immunodeficient or immunosuppressed due to an Epstein-Barr virus infection.

33. The composition according to claim 28, wherein said viral infection of herpes groups is a herpes simplex virus infection.

34. The method according to claim 30, wherein said viral infection of herpes groups is a herpes simplex virus infection.

REMARKS

Favorable reconsideration is respectfully requested in view of the foregoing amendments and the following remarks.

Initially, Applicants and their undersigned representative wish to express their sincere appreciation to the Examiner for his courtesy and helpful comments made during the personal and telephone interviews.

Claims 5-11 have been cancelled without prejudice, and replaced with new claims 12-34. The new claims have been added to more particularly define the present invention and to further protect specific embodiments of the present invention. Support for the new claims is readily apparent from the teachings of the specification and the original claims. Specific support for the

new claims can be found on pages 7, 8 and 10 of the specification.

In particular, support for the definition of "herpes groups" (i.e. herpes family) is found on, for example, page 7, last line, and page 8, lines 8-9, of the specification. Further support for the phrase *"viral infections of* (i.e. belonging to) *herpes groups"* can be found in EMBODIMENT 4 on page 13, lines 16-22, of the specification (i.e. "*the effect of the lymphocytes against the viral infections caused by EB viruses became evident*"). Also, the following passage in EMBODIMENT 3 on page 13, lines 9-10, of the specification also support this newly added claim phrase.

> "*Consequently, the affected focus in the patient's eye due to the herpes simplex infections was cured. The result thereof revealed that the lymphocytes are efficacious against viral infections caused by EB viruses*"

With regard to the rejection of claim 11 under 35 USC § 112, first paragraph, this rejection has been rendered moot by the cancellation of claim 11.

With regard to the rejection of claims 5-11 under 35 USC § 103(a) as being unpatentable over Ochoa et al. (USP 5,443,983) in view of Rosenberg, this rejection has been overcome by the cancellation of claim 5-11.

With respect to the Examiner's arguments as applied to the new claims, Applicants have the following comments.

To establish a *prima facie* case of obviousness under U.S. practice, the cited references in combination must teach or suggest the invention as a whole including all the limitations of the claims.

As for composition claim 12 and method claim 13, and the claims dependent thereon, Applicants believe that the cited references in combination do not render obvious these claims

since the cited references failed to teach or suggest the claim limitations ***"autologous"*** and ***"said activated autologous lymphocytes being obtained by culturing autologous lymphocytes derived from a virally infected patient or an immunodeficient or immunosuppressed patient"***.

As stated in Applicant's response of November 5, 2001, the presently claimed composition comprises "autologous" lymphocytes, which are lymphocytes that are derived from the same patient who ultimately undergoes treatment with such lymphocytes. None of the cited references including Ochoa et al. (USP 5,443,983) discloses or suggests a composition comprising activated "autologous" lymphocytes. Although the cited Ochoa et al '983 discloses an example in which peripheral blood lymphocytes (PBLs) are collected from the twin brother of a patient (see Example 4 of the reference), the twin brothers are identical in genetics, but have different activated lymphocytes.

Ochoa et al. show only a preparation of lymphocytes against tumors and does not at all teach or suggest activated ***"autologous"*** lymphocytes. The cited reference only discusses generally the preparation of lymphocytes using anti-CD3 antibody and IL-2 but does not at all teach or suggest the limitation "autologous". Also, the reference of Rosenberg is related only to lymphocytes prepared using IL-2 only and does not at all teach or suggest the claim limitation "autologous".

Also, as stated earlier, the cited references fail to teach or suggest the claimed limitation ***"said activated autologous lymphocytes being obtained by culturing autologous lymphocytes derived from a virally infected patient or an immunodeficient or immunosuppressed patient"***. Rosenberg merely teaches the generalities of IL-2 applicable for medical treatment of immune dysfunctional diseases but fail to specifically disclose that the source of the autologous

lymphocytes is derived from a virally infected patient or an immunodeficient or immunosuppressed patient. The reference of Ochoa et al. also fails to teach or suggest this specific claim limitation.

Applicants wish to emphasize and the Examiner to understand that an important characteristic feature of the present invention is that the activated lymphocytes used in the present invention are derived from an virally infected, immunodeficient or immunosuppressed patient and not a healthy person. In this field of art, it has been common knowledge that lymphocytes, as a curative medicine to be provided for an immunocompromised patient, must be taken from a healthy person. Up until the present invention, it could not be imagined that lymphocytes of immunocompromised patients could be provided for the patient himself or herself. The present invention has caused a sensation in the medical world in Japan and this discovery has been found to be revolutionary in this field of art.

With regard to claim 14 and the claims dependent thereon, both Ochoa et al. and Rosenberg fail to render obvious these claims since the cited references fail to teach or suggest not only the claim limitation ***"said activated autologous lymphocytes being obtained by culturing autologous lymphocytes derived from a virally infected patient or an immunodeficient or immunosuppressed patient"*** but also the limitations ***"for preventing or treating viral infections of herpes groups"*** and ***"administering said activated autologous lymphocytes to said patient from which said autologous lymphocytes were derived".*** The Examiner has failed in the present and past Official Actions to cite a passage in Ochoa et al. and Rosenberg which teach or suggest such a claim limitation.

Also, with regard to the dependent claims, Applicants note that the Examiner has not

specifically pointed out in the present and past Official Actions where in each of the cited references, the limitations of the dependent claims are presented. Applicants respectfully request the Examiner to cite location of these dependent claim limitations in the next Official Action or withdraw this 103 rejection based on these limitations.

Applicants also wish to note that claims 28-34 have been presented to direct to compositions, and methods of making and using thereof wherein the virally infected patient or the immunodeficient or immunosuppressed patient is caused by a viral infection of herpes groups or specifically, an Epstein-Barr or herpes simplex virus infection

Another requirement to establish a *prima facie* case of obviousness under U.S. practice, the cited references must provide a basis for modifying the teachings of the prior art. Further, one skilled in the art must also reasonably expect that the modification would be successful based upon the teachings and suggestions of the prior art. Here, Ochoa et al. show only a preparation of lymphocytes against tumors and does not at all teach or suggest that activated autologous lymphocytes have antiviral activity. The cited reference only discusses generally the preparation of lymphocytes using anti-CD3 antibody and IL-2 which have already been reviewed in the Background section of the specification. In other words, the teachings provided by Ochoa et al. is substantially identical with JP03-80076A noted in the specification.

Also, as stated earlier, Rosenberg merely describes the generalities of IL-2 applicable for medical treatment of immune dysfunctional diseases but does not prove whether the lymphocytes prepared using only IL-2 are effective on viral infections or not. It is important to note that the cited reference Rosenberg only mentions viral infections in passing (see column 4, lines 49-54), and does not contain any data or teachings which prove and enable one skilled in the art to make

and use activated autologous lymphocytes against viral infections. In fact, the reference, Nature Medicine, 1(4) pp. 330-336 (1996), discloses that lymphocytes prepared using IL-2 were not effective in remedying HIV infections, as stated on page 4 of the specification. Thus, there is clearly no teaching or suggestion in the cited references that activated autologous lymphocytes as recited in the present invention would be effective against viral infections. As a result, the teachings and suggestions of both of these references does not create a reasonable expectation of success to one skilled in the art that activated autologous lymphocytes being derived from a culture medium comprising autologous lymphocytes, anti-CD3 antibodies in a solid phase and interleukin-2 would be very effective for viral infections.

Therefore, since a *prima facie* case of obviousness cannot be established for new claims 12-14 and the claims dependent thereon, a rejection under 35 USC § 103 based on previously cited references, Ochoa et al. and Rosenberg, would be untenable.

In addition, although the Examiner expressed during the personal interview that the Applicants have not shown that their invention has a widely available antiviral spectrum, Applicants believes that the Examiner has not fully appreciate the significance of expermental data set forth in the specification. At present, there are antiviral preparations such as Acyclovir and Gancyclovir. These preparations are effective against a herpes simplex virus and cytomegalovirus. However, these preparations have little effectiveness against the Epstein-Barr (EB) virus. In light of the fact that there is no medicine currently with a curative effect on the EB virus, a person skilled in this field could understand that a medicine with a curative effect on the EB virus should be effective against the other viral infections and ***in particular viral infections of herpes groups***. Accordingly, a correct evaluation of this invention cannot be made unless the Examiner correctly

understand that the EB virus is extremely hard to cure.

Also, it is known that herpes simplex virus develops resistance to Acyclovir and Gancyclovir and the patients in the Examples of the specification was affected by a herpes simplex virus resistant to Acyclovir. The experimental results in the specification clearly show that the compositions and methods of the present invention are also effective against herpes simplex virus infections.

It should also be noted that herpesvirus is not a large genus. To date, there are 8 known human Herpesviruses including herpes simplex virus **(HSV)**, cytomegalovirus **(CMV)**, varicella zoster **(VZV)**, and Epstein Barr virus **(EBV)**. The family is divided into 3 sub-families:

Alphaherpesvirinae:	
Simplexvirus	human herpesvirus 1, 2 (HSV-1, HSV-2)
Varicellovirus	human herpesvirus 3 (VZV)
Betaherpesvirinae:	
Cytomegalovirus	human herpesvirus 5 (HCMV)
Muromegalovirus	mouse cytomegalovirus 1
Roseolovirus	human herpesvirus 6, 7 (HHV-6, HHV-7)
Gammaherpesvirinae:	
Lymphocryptovirus	human herpesvirus 4 (EBV)
Rhadinovirus	human herpesvirus 8 (HHV-8)

Herpesviruses have an envelope surrounding an icosahedral capsid, approximately 100nm in diameter, which contains the dsDNA genome. The herpes virus capsid is an icosahedron of triangulation number T = 16. There are 12 pentavalent capsomers (one at each apex) and 150 hexavalent capsomers. Each capsomer has a deep central indentation. There is an envelope that

surrounds the nucleocapsid and it is derived from the inner nuclear membrane of the host cell. Virus-encoded glycoproteins are incorporated into the virion envelope and are visible as "spikes" that project from its surface. Between the capsid and the envelope is an ill-defined layer of proteins, collectively known as the tegument.

Thus, given the small size of the genus (herpesvirus), the common attributes of the species in the genus (noted above) and the Examples of the specification showing efficacy of the claimed composition and method against the Epstein-Barr virus and herpes simplex virus infections, Applicants strongly believe that the claimed invention is patentable under U.S. practice.

In further support of the Applicants' arguments noted above, Applicants have submitted the reference entitled "Adoptive Immunotherapy for Interstitial Pneumonia Associated with Cytomegalovirus Infection". This reference demonstrates that the experimental results directed to the Epstein-Barr and herpes simplex viruses can be extrapolated to support the effectiveness of the present invention for other viral infections and in particular viral infection of herpes groups.

In view of the foregoing amendments and remarks, it is respectfully submitted that the Application is now in condition for allowance. Such action is thus respectfully solicited.

If, however, the Examiner has any suggestions for expediting allowance of the application or believes that direct communication with Applicants' attorney will advance the prosecution of

this case, the Examiner is invited to contact the undersigned at the telephone number below.

Respectfully submitted,

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Adoptive Immunotherapy for Interstitial Pneumonia Associated with Cytomegalovirus Infection

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Primary infection with cytomegalovirus (CMV) usually follows a benign course, but the virus remains latent or persistent in the host cell. Under immunosuppressive conditions, latent or persistent infection can be reactivated to produce a wide variety of clinical manifestations. Some antiviral agents that are active against virus-specific metabolic processes without producing cytotoxicity have become available. Unfortunately, no successful treatment of CMV infection in children has yet been developed.

Adoptive transfer of antigen-specific cytotoxic T lymphocytes (CTLs) offers safe and effective therapy for certain viral infections. Walter et al. [1] provided important evidence that infusion of donor-derived $CD8^+$ cytotoxic T cell clones specific for CMV can promptly reconstitute cellular immunity against CMV in recipients of allogenic bone marrow, thus reducing the risk of morbidity and mortality related to viral infection. However, the persistence of transferred $CD8^+$ cytotoxic cells was prompted by the recovery of the response of $CD4^+$ CMV-specific helper T cells.

The infusion of virus-specific polyclonal T cell lines containing both $CD4^+$ and $CD8^+$ cells has been successfully used to control infection with Epstein-Barr virus (EBV) and related lymphoproliferative disorders in recipients of allogenic bone marrow [2]. Heslop et al. [3] reported that infusions of CTLs not only restored cellular immune response against EBV but also established that populations of CTL precursors could response to the challenge

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with virus for as long as 18 months. However, Locatelli et al. [4] suggested that donor-derived polyclonal T cell lines enriched with CMV-reactive T cells might be also useful for the reconstitution of immunity.

We describe a patient who had interstitial pneumonia associated with CMV infection and whose clinical condition improved after treatment with primary adoptive immunotherapy. This study was approved by the Committee on Clinical Evaluation at Sapporo Medical University. Informed consent was obtained from the patient's parents.

A 17-month-old girl was admitted to the hospital because of cough and dyspnea. A chest roentgenogram demonstrated interstitial pneumonic shadow, and she was treated with prednisolone. The girl was admitted again 2 months later with another episode of interstitial pneumonia. At this time, serum IgG and IgM antibodies to CMV were detected, and CMV was isolated from sputum and urine. CMV DNA was also detected in her peripheral blood mononuclear cells by PCR. Although reactivation of CMV was suspected serologically, the $CD4^+/CD8^+$ cell ratio and the number of $CD4^+$ cells were within the normal range.

The patient did not receive therapy with immunoglobulin at this time. Since there have not been adequate studies on the use of ganciclovir or foscarnet for the treatment of CMV infection in nonimmunocompromised infants and children, the patient did not receive these drugs as treatment. Her peripheral blood mononuclear cells were separated from 20 mL of blood and cultured with immobilized monoclonal antibody to CD3 cells and human recombinant interleukin 2 (rIL-2). Rapid proliferation of T lymphocytes was obtained by this procedure [5]. Cultured T lymphocytes were then transferred to a gas-permeable culture bag, and culture was continued for an additional 8 days (the volume of the medium was increased over this time). The patient's T cells increased about 2,000-fold during 2 weeks of culture. The final T lymphocyte population contained about 30% $CD4^+$ T lymphocytes and 60% $CD8^+$ T lymphocytes. The cytotoxic activity of the $CD4^+$ T lymphocytes was as strong as that of the $CD8^+$ T lymphocytes [6].

T lymphocytes generated from the patient's peripheral blood were administered intravenously to the patient in six doses (4.3×10^9 to 1.1×10^9 cells), each given 1–2 weeks apart. No major toxic effects were observed after six infusions of a total of 4.03×10^{10} cells. However, after therapy was completed, a chest radiograph showed resolution of infiltrates, CMV was not isolated from sputum, and CMV DNA was not detected from peripheral blood mononuclear cells. The patient's condition improved, and she was discharged from the hospital in good condition.

CTLs and natural killer cells have complementary roles in the recovery from CMV infection. CMV infection may also affect the activation of cellular immunity and the secretion of IL-2 that stimulate natural killer cells with cytotoxic activity. We studied the relationship between CMV infection and cytokines [7, 8]. In the active phase of CMV infection, titers of cell-free soluble IL-2 receptor were correlated with serum levels of liver enzymes in some cases. It is likely that expression of viral genome on T lymphocytes as well as activities of some cytokines are associated with active CMV infection.

The patient's clinical symptoms decreased, her abnormal laboratory findings resolved, and CMV disappeared from clinical specimens after she received treatment with primary adoptive immunotherapy. The virus-specific $CD8^+$ cytotoxic T cells and $CD4^+$ helper or cytotoxic T cells may be amplified and used to control CMV infection. Although the exact mechanisms of anti-CMV effects have not been clarified, adoptive immunotherapy with immobilized anti-CD3 antibody-activated T lymphocytes might be clinically effective for CMV infection in children.

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References

1. Walter EA, Greenberg PD, Gilbert MJ, et al. Reconstitution of cellular immunity against cytomegalovirus in recipients of allogenic bone marrow by transfer of T-cell clones from the donor. N Engl J Med 1995;333: 1038–44.
2. Rooney CM, Smith CA, Ng CY, et al. Use of gene-modified virus-specific T lymphocytes to control Epstein-Barr-virus-related lymphoproliferation. Lancet 1995;345:9–13.
3. Heslop HE, Ng CYC, Li C, et al. Long-term restoration of immunity against Epstein-Barr virus infection by adoptive transfer of gene-modified virus-specific T lymphocytes. Nature Medicine 1996;2:551–5.
4. Locatelli F, Maccario R, Gerna G. Anticytomegalovirus T-cell clones [letter]. N Engl J Med 1996;334:601.
5. Sekine T, Shiraiwa H, Yamazaki T, Tobisu K, Kakizoe T. A feasible method for expansion of peripheral blood lymphocytes by culture with immobilized anti-CD3 monoclonal antibody and interleukin-2 for use in adoptive immunotherapy of cancer patients. Biomed Pharmacother 1993;47: 73–8.
6. Konomi Y, Sekine T, Takayama T, Fuji M, Tanaka T. Cytotoxic activity of CD4+ T cells against autologous tumor cells. Jpn J Cancer Res 1995; 86:854–60.
7. Asanuma H, Numazaki K, Nagata N, Chiba S. Cytokine response and polymerase chain reaction study of peripheral blood mononuclear cells in infants with human cytomegalovirus infection. FEMS Immunol Med Microbiol 1995;12:153–8.
8. Numazaki K, Asanuma H, Hotsubo T, Chiba S. Anti-human cytomegalovirus effects of breast milk [letter]. J Infect Dis 1996;174:444.

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4. Locatelli F, Maccario R, Gerna G. Anticytomegalovirus T-cell clones [letter]. N Engl J Med 1996;334:601.
5. Sekine T, Shiraiwa H, Yamazaki T, Tobisu K, Kakizoe T. A feasible method for expansion of peripheral blood lymphocytes by culture with immobilized anti-CD3 monoclonal antibody and interleukin-2 for use in adoptive immunotherapy of cancer patients. Biomed Pharmacother 1993;47: 73–8.
6. Konomi Y, Sekine T, Takayama T, Fuji M, Tanaka T. Cytotoxic activity of CD4+ T cells against autologous tumor cells. Jpn J Cancer Res 1995; 86:854–60.
7. Asanuma H, Numazaki K, Nagata N, Chiba S. Cytokine response and polymerase chain reaction study of peripheral blood mononuclear cells in infants with human cytomegalovirus infection. FEMS Immunol Med Microbiol 1995;12:153–8.
8. Numazaki K, Asanuma H, Hotsubo T, Chiba S. Anti-human cytomegalovirus effects of breast milk [letter]. J Infect Dis 1996;174:444.